The health challenge of the carbapenemase-producing *Enterobacteriaceae*

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Bacterial multiresistance to antibiotics has become a major source of concern for public health in the last years. The severity of this threat has increased by the fact that research for new antibiotic agents is currently stalled. Carbapenems are members of the ß-lactam family, the latest developed molecules that posses the broadest spectrum of antimicrobial activity and they are crucial for treating life-threatening infections.
Carbapenemase-producing Enterobacteriaceae (CPE) are progressively spreading throughout the world. The production of carbapenem-hydrolysing ß-lactamases confers resistance to almost all ß-lactams and, in most cases; they also carry other non-ß-lactam resistance mechanisms leading to multidrug resistant isolates. Unfortunately, the prevalence of CPE has increased during the past 10 years, seriously compromising the therapeutic armamentarium and poses a challenge in the treatment and control of these infections.

The aim of this review is to analyze the CPE problematic, to discuss their distinctive traits and to evaluate the current available detection methods, treatment options and finally, decide which one is the best way forward to address this alarming situation.

Figure 1. NDM producing *Escherichia coli* http://www.bioquell.ie/technology/microbiology/new-delhi-metallo-beta-lactamase-1-ndm-1/

Carbapenemases

Carbapenemases are encoded mostly by *bla* genes carried on mobile elements (e.g. plasmids and/or integrons) that facilitates their horizontal spread among different Gram-negative species.
Three types of carbapenemases have been identified:

-Class A carbapenemases (KPC, IMI, SME and GES types) are often carried by *Klebsiella pneumoniae* isolates, but they have been found also in other enterobacterial species like *Klebsiella oxytoca, Salmonella enterica, Proteus mirabilis* or *Serratia marcescens* (Figure 2). KPC producers are the most clinically common, they are able to hydrolyze all ß-lactams and most of the other antibiotic families [2].

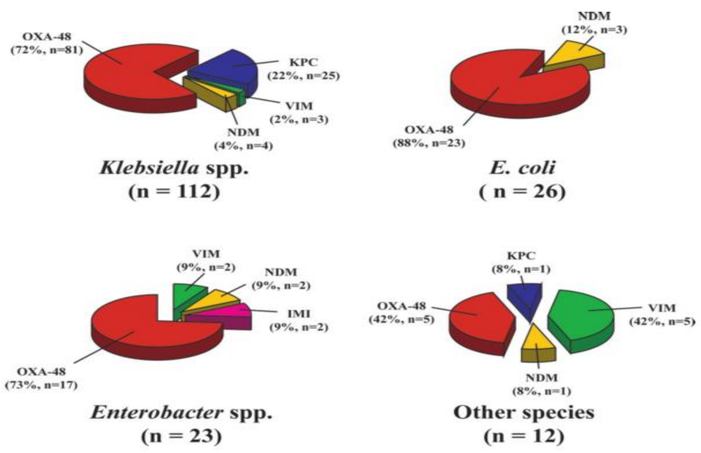

Figure 2. Dortet et *al.* Characterized 172 carbapenemase producers using DNA microarray + sequencing. 65% of CPE were *K.pneumoniae*, 15% were *E.coli*, 13% were *Enterobacter spp.* and 7% belonged to other enterobacterial species. The identified carbapenemases were of the OXA-48 (72%), KPC (15%), NDM (6%), VIM (6%), and IMI types (1%) [1].

-Class B or metallo- ß-lactamases (IMP, VIM, SPM and NDM types) exhibit a broad spectrum of hydrolytic activity, including all penicilins, cephalosporins and carbapenems. Plasmid encoding NDM genes can harbor a really high number of associated resistances, they are frequently acquired by *K.pneumoniae* and *Escherichia coli* (Figure 2), two of the most common pathogens, and they arise from high density and low-income zones like the sub-Indian continent or Pakistan. These traits have made NDM enzymes of extraordinary concern for health authorities and now the focus is on preventing their spread [2].

- Class D carbapenemases are represented by OXA-48, which is exclusive for *Enterobacteriaceae.* They are well distributed among all enterobacterial species with high prevalence (Figure 2). OXA-48 enzymes have a weak carbapenemase activity, therefore, they may go unnoticed for susceptibility tests, complicating detection and control measures and making dissemination much easier [2].

Treatment

According to recent data from the Centers for Disease Control and Prevention in the United States, the percentage of CPE increased almost ten times from 2001 to 2011, and it also has been shown that patients with these infections experience fatality rates of 50 %. An important question which still unanswered among clinicians is whether combination or monotherapy antibiotic regimens are more effective. Tzouvelekis et *al.* [4] performed a study to evaluate different antibiotic regimens (Figure 4). Results show that treatment failure was more common in patients who were treated with monotherapy (colistin, tigecycline and aminoglycoside). However, lower fatality rates are shown in patients treated with combination regimes. This may be due to synergic effect of the drugs and lesser probabilities to acquire resistances.

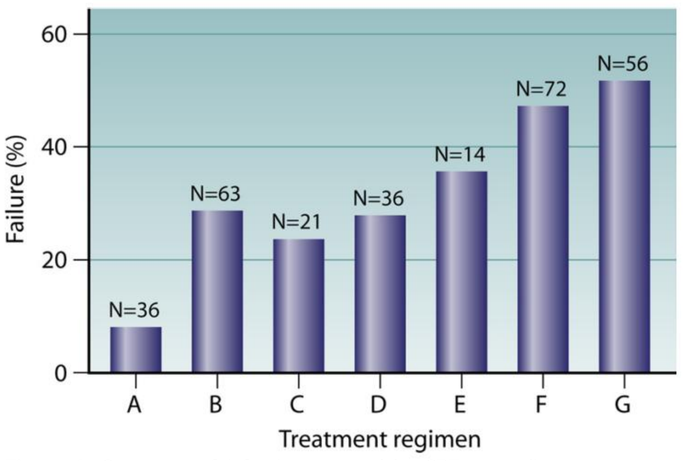

Figure 4. Outcomes of infections caused by CPE, according to treatment regimen. Regimen A, combination therapy with ≥2 active drugs, one of which was a carbapenem; regimen B, combination therapy with ≥2 active drugs, not including a carbapenem; regimen C, monotherapy with an aminoglycoside; regimen D, monotherapy with a carbapenem; regimen E, monotherapy with tigecycline; regimen F, monotherapy with colistin; regimen G, inappropriate therapy [4].

Based on this study, it seems that carbapenems retain some therapeutic efficacy against CPE infections. According to these results, combined therapy with a carbapenem and another active drug (colistin or aminoglycoside) reduces fatality rates, and therefore, could be the best treatment strategy. Nevertheless, caution must be taken because resistances, although less probably, may still arise [5].

Detection

There is no universal method able to detect all types of carbapenemase producers with high sensitivity and specificity. Detection of CPE is first based on an analysis of susceptibility testing results, but automated systems may not reliably detect all types of carbapenemase producers and here is when first discrepancies arise. CLSI and EUCAST have set some breakpoints for susceptibility testing, but some studies conclude that these breakpoints should be lowered and other characteristics should be considered in order to detect any slight decrease in susceptibility to carbapenems to obtain more accurate results [3,5].
If the susceptibility test results from an isolate are positive, carbapenemase production have to be detected. A series of phenotypic and molecular tests for detection of carbapenemase activity are discussed in Table 1:

Table 1. Main characteristics of carbapenemase-producing *Enterobacteriaceae* detection methods

	Phenotypic methods				Molecular methods	
Test parameters	MHT	UV spectrophotometry	MALDI-TOF MS	Carba NP test	PCR techniques	DNA Microarray
Efficiency (%)						
Sensitivity	100	100	100	100-80	100	98.8
Specificity	87	100	100	100	100	100
Other characteristics						
Rapidity (h)	16-24	12-24	2-4	< 2	24-48	8-24
Cost[1]	$	$	$	$	$$	$$$
Expertise needs[2]	++	+++	+++	+	++	++
Carbapenemase detected	KPC, OXA-48 and NDM (+Zn)	All types (No discrimination)	All types	All types (FNR[3]for OXA-48,IMP and NDM)	All types (No novel)	KPC,VIM, NDM,IMP, IMI and OXA

[1] The number of $'s correlates with the effective (relative) price of the test
[2] The number of +'s correlates with the expertise and training needed to perform and interpret the test
[3] FNR, false negative results

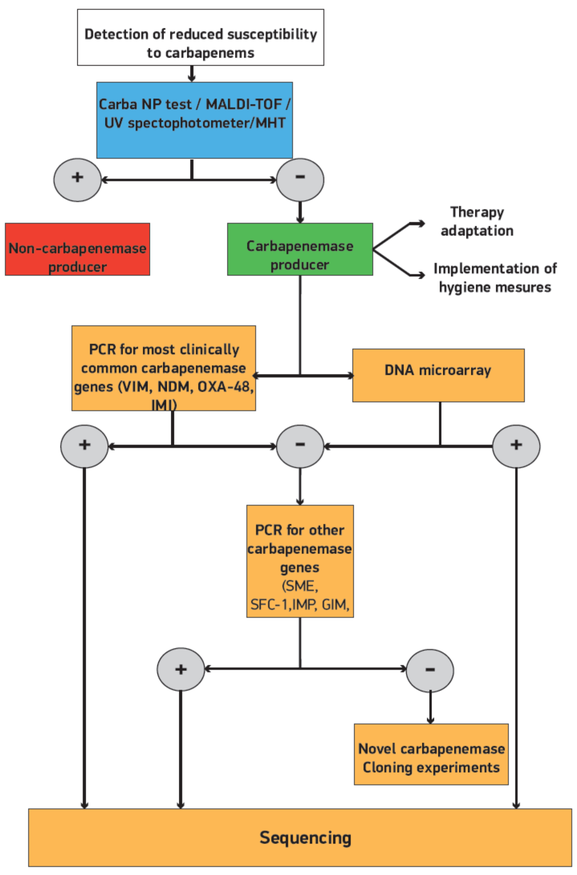

Figure 3. Flowchart for detection and characterization of carbapenemase producers among *Enterobacteriaceae.* Molecular techniques (PCR, DNA microarray, sequencing) only for precise identification of carbapenemase genes. This step may be followed only in university hospitals or large microbiology laboratories [1]

Conclusions

The emergence of CPE as a substantial threat to health care should prompt health authorities to formulate a preparedness plan ready for implementation, ensuring early detection with standardized methods and accurate treatment. In this area, treatment with combination therapy seems the best option, although new resistances may still arise. A future alternative might be the development of new molecules targeting bacterial metabolism. Furthermore, inhibition of carbapenemases through the interaction of molecules with the carbapenemase active site has also been suggested.

However, the fast transmission of these resistances and the genetic plasticity of bacteria make this challenge even more unlikely.

References

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